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BRIEF REPORT

Rotavirus Vaccine Take in Infants Is Associated With Secretor Status

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Rotaviruses bind to enterocytes in a genotype-specific manner via histo-blood group antigens (HBGAs), which are also detectable in saliva. We evaluated antirotavirus immunoglobulin A seroconversion ("vaccine take") among 166 Ghanaian infants after 2–3 doses of G1P[8] rotavirus vaccine during a vaccine trial, by HBGA status from saliva collected at age 4.1 years. Only secretor status was associated with seroconversion: 41% seroconversion for secretors vs 13% for nonsecretors; relative risk, 3.2 (95% confidence interval, 1.2–8.1; $P = .016$). Neither Lewis antigen nor salivary antigen blood type was associated with seroconversion. Likelihood of "take" for any particular rotavirus vaccine may differ across populations based on HBGAs.

Keywords. secretor; rotavirus; vaccine; *FUT2*; Lewis antigen.

Recent laboratory advancements have transformed the understanding of host–pathogen interaction for rotaviruses, elucidating the required expression of specific histo-blood group antigens (HBGAs) on the gastrointestinal mucosa for rotavirus binding [1–4]. Rotaviruses are designated by genes encoding the VP7 (termed G genotype) and VP4 (P genotype) proteins. VP4 is cleaved to VP8*, which appears to bind to particular HBGAs in a genotype–specific manner. Translational epidemiological studies support the concept that there are differential disease risks from specific P genotypes, by host HBGAs [5–9]. Both rotavirus vaccines in wide use have the human rotavirus P[8] genotype, and understanding whether HBGAs restrict vaccine take and contribute to the differences observed in rotavirus vaccine performance measured across different settings is an important goal. We previously evaluated the immunogenicity of Rotarix vaccine (GSK Biologics, Rixensart, Belgium; RV1) in Ghanaian infants [10]. Here we investigated whether HBGAs of trial participants, measured in saliva, predicted take of RV1.

METHODS

In the original trial (NCT015751) conducted in 2012–2013, healthy infants were consented and randomized into 3 arms to receive RV1: arm 1 (RV1 at ages 6 and 10 weeks); arm 2 (RV1 at 10 and 14 weeks); arm 3 (RV1 at 6, 10 and 14 weeks). Serum samples were obtained just before RV1 dose 1 and 1–2 months after the last dose [10]. Infants who were negative for serum antirotavirus immunoglobulin A (IgA) antibody just before dose 1 were included in per-protocol results. The follow-on study with saliva collection was approved by institutional review boards of participating institutions. Parents of trial participants were approached August 2016–February 2017 for their child's participation (median age, 4.1 years [interquartile range, 4.0–4.4]); saliva was collected from the child if consent was given (Supplementary Materials). Saliva study enrollment was prioritized by arm (arm 3 > arm 2 > arm 1) because the higher seroconversion rates in arms 3 and 2 provided greater power to detect an association with HBGA status if one existed [10].

The child's HBGA phenotype was determined by testing saliva for H antigen, Lewis a and b antigens, and A and B blood group antigens using enzyme immunoassays and lectin and anti-HBGA antibodies (Supplementary Materials) [11]. Initial phenotype categories were secretor positive, low, or negative; Lewis positive, low, or negative; and blood group O, A, B, or AB. Phenotype results were used to select a subset of samples for DNA extraction and genotyping. Twelve initial samples had fucosyltransferase 2 (*FUT2*, the so-called secretor gene) genotyping based on G428A nonsense single-nucleotide polymorphism, and then 76 additional samples were selected for *FUT2* genotyping: all secretor negatives (n = 26), all low secretors (n = 18), samples with inconsistent HBGA results (n = 3), and secretors from arm 2 and 3 that were Lewis low (n = 10) or were Lewis negative and blood type O (n = 19). The 21 samples selected for *FUT3* (the so-called Lewis gene) genotyping were those from arm 2 and arm 3 that were negative for all HBGAs assessed (n = 4), Lewis low (n = 11), a random selection of those Lewis negative and blood type O (n = 2), and a random selection of those Lewis positive and blood type O (n = 4) (Supplementary Materials).

Except for testing of the initial 12 samples, the laboratory that performed genotyping was different from the laboratory that performed phenotyping, and laboratory staff that performed

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genotyping were blinded to phenotype results and the reasons for requesting genotyping. Laboratory staff testing saliva were blinded to serum results.

During the original trial, antirotavirus IgA antibody concentrations in serum had been determined using enzyme immunoassay [10]. Preexisting antirotavirus immunoglobulin G (IgG) antibody concentration, presumed to be maternally derived, had been measured in serum collected just before RV1 dose 1 [10]. Seroconversion was defined as antirotavirus IgA antibodies ≥20 U/mL after RV1 doses (at 18 weeks in arm 3 and arm 2 infants; at 14 weeks in arm 1 infants) among infants seronegative (IgA <20 U/mL) just before dose 1.

Relative risks (RRs) of seroconversion, stratified by secretor (positive vs negative), Lewis (positive vs negative), and blood group by salivary antigen, were estimated using binomial log-linear regression. For the main analysis, the child's status was categorized based on all available information (HBGA phenotype, plus *FUT2* and/or *FUT3* genotype); genotype was used over phenotype if results were discrepant. Subjects whose Lewis antigen status could only be based on phenotype and were in the Lewis low category were classified as Lewis negative. Secondary analyses were performed using only phenotype results. Variables planned a priori to assess for possible confounding were randomization arm (arm 3 or 2 vs arm 1), antirotavirus IgG antibody concentration before dose 1 (by quartile), nutritional status at age 14 weeks by *z* scores, and exposure to season of higher wild-type rotavirus circulation (mid-December 2012–February 2013, as determined by local diarrhea surveillance and original trial data) after the serum collection pre-RV1 dose 1 and before serum collection post-RV1. Confounding was assessed by backward elimination; factors whose elimination changed the risk ratio of the variable of interest by ≥10% were retained.

Genotyping data from surveillance demonstrated that genotypes G1P[8] and G2P[4] predominated during late 2012–early 2013; P[6] strains were not detected (unpublished data).

RESULTS

Saliva was collected from 166 children: 57% (82/143) of arm 3, 28% (39/139) of arm 2, and 32% (45/142) of arm 1 participants. The phenotype/genotype concordance was 87% (73/84) for secretor status and 62% (8/13) for Lewis status (Supplementary Materials). Overall, 81% (135/166) of infants were secretors and 19% (31/166) were nonsecretors; 57% (95/166) were Lewis positive, 43% (71/166) were Lewis negative. Among secretors, by salivary antigen assay, 52% (70/135) were blood type O, 30% (40/135) type B, 16% (22/135) type A, and 2% (3/135) type AB.

Overall, 41% of secretors seroconverted compared with 13% of nonsecretors (Table 1). In the model that included only secretor status, secretors were 3.2 (95% confidence interval [CI], 1.2–8.1; $P = .016$) times more likely to seroconvert than nonsecretors (Table 2). Neither arm, Lewis status, nor any other factor evaluated were confounders (RR for Lewis positive compared with Lewis negative, 0.9 [95% CI, .6–1.4]; $P = .65$); Lewis status was also not an effect modifier (interaction term $P = .37$). Results were similar when secretor and Lewis status were based only on phenotype for all infants (RR secretor vs nonsecretor, 3.8 [95% CI, 1.3–11.2]; RR Lewis positive vs negative, 1.0 [95% CI, .7–1.5]; interaction term $P = .11$) and when all information was used but the 16 phenotypically "Lewis low" subjects were excluded rather than categorized as Lewis negative, or categorized as Lewis positive (Supplementary Materials). Exposure to period of higher wild-type rotavirus circulation was not found to be a confounder or effect modifier. Using only the 84 children that had *FUT2* genotype results available (note: most selected for genotyping had low *Ulex europaeus* agglutinin I (UEA-1) assay optical densities), the RRs for seroconversion for secretors vs nonsecretors were similar when those subjects were classified as secretor or nonsecretor based only on genotype result, or when based only on phenotype results (Supplementary Materials).

Including only secretors in the model, there was no statistically significant difference in seroconversion by salivary blood group antigen. However, the lower likelihood for seroconversion among type O vs type B just missed statistical significance (RR, 0.7 [95% CI, .4–1.0]) (Table 2). In these models, the RR for Lewis positive vs negative ranged from 1.0 to 1.2 and was not statistically significant. The RR for seroconversion was not statistically different among phenotypic "high" vs "low" secretors (RR, 1.3 [95% CI, .7–2.3]; $P = .38$); few children were "low" secretors.

Table 1. Proportion of Infants That Seroconverted by Secretor Status, Lewis Status, Salivary Antigen Blood Group Type, and Study Arm

Category	Seroconverted/Total, No. (%)
Arms combined (n = 166)	
Nonsecretor (n = 31; 19% of total)	4/31 (13)
Lewis positive	2/22 (9)
Lewis negative	2/9 (22)
Secretor (n = 135; 81% of total)	55/135 (41)
Lewis positive	29/73 (40)
Lewis negative	26/62 (42)
Type O	25/70 (36)
Type B	20/40 (50)
Type A	9/22 (41)
Type AB	1/3 (33)
Arms 2 + 3 (n = 121)	
Nonsecretor (n = 23; 19%)	4/23 (17)
Secretor (n = 98; 81%)	46/98 (47)
Arm 1 (n = 45)	
Nonsecretor (n = 8; 18%)	0/8 (0)
Secretor (n = 37; 82%)	9/37 (24)

Table 2. Relative Risks of Seroconversion by Secretor/Histo-Blood Group Antigen Status, From Regression Models

	Using Phenotype Plus Available Genotype Results (n = 166)		Using Phenotype Results Only (n = 166)		Using Secretors Only[a] (n = 135)	
Secretor/HBGA Status	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value
Secretor positive vs negative	3.2 (1.2–8.1)[b]	.016	3.8 (1.3–11.2)	.016	…	
Lewis positive vs negative	0.9 (.6–1.4)	.65	1.0 (.7–1.5)	.95	0.9 (.6–1.4)	.64
Salivary ABO blood group						
O vs non-O	…		…		0.8 (.5–1.2)	.22
O vs A	…		…		0.8 (.4–1.4)	.40
O vs B	…		…		0.7 (.4–1.0)	.055
O vs AB	…		…		0.8 (.2–4.4)	.84

Abbreviations: CI, confidence interval; HBGA, histo-blood group antigen; RR, relative risk.

[a]Using phenotype plus available genotype results.

[b]RR, 3.1 (95% CI, 1.2–7.9), *P* = .018, in model adjusted for Lewis status, study arm, exposure to period of higher wild-type rotavirus circulation, antirotavirus immunoglobulin G pre-RV1 dose 1, and height for age at 14 weeks.

Among subjects who seroconverted, serum antirotavirus IgA concentrations were not statistically different between different subject groups (ie, secretor positive vs negative; Lewis positive vs negative overall; Lewis positive vs negative among secretors only).

DISCUSSION

We investigated the correlation between HBGAs and rotavirus infection (defined by seroconversion) shortly after a standardized exposure to G1P[8] vaccine strain among rotavirus-naive infants, while accounting for other factors. Our data support the hypothesis that susceptibility to G1P[8] infection, and therefore take of RV1, is specifically associated with secretor status. Differences in HBGA distributions across populations may contribute to the differences in results on vaccine take and efficacy across different regions.

Our findings provide additional in vivo evidence supporting the hypothesis that susceptibility to P[8] infection is associated with secretor status. In a similar study among Pakistani infants, with the same laboratories performing the serologic and salivary phenotyping assays as in this study, infants who were nonsecretors also were not absolutely restricted from seroresponse but had the lowest rate of seroconversion (19%) following RV1 exposure [11]. Also similar to our Ghana findings, in the Pakistan evaluation, Lewis status was not independently associated with seroconversion at a statistically significant level, although the numbers of Lewis-negative children in that study were low. In a study of Nicaraguan infants aged approximately 8 weeks, most of whom had detectable antirotavirus IgA at time of vaccination, increased antirotavirus titer or seroconversion following RV1 dose 1 was detected in a statistically higher proportion of secretors (24%) vs nonsecretors (8%), in univariate assessment [12]. Other studies, with some population diversity, compared secretor status of children with rotavirus *disease* from P[8] strains with the general population and found that those with P[8] disease were significantly more likely to be secretors [5–8]. Based on analysis of saliva samples collected from 275 Bangladeshi children aged 1–2 years who had been under active surveillance as the unvaccinated cohort in a vaccine trial, researchers found an overall increased risk of rotavirus disease to age 1 year among phenotypic secretors vs nonsecretors [13]. This difference, however, was due to differences in risk of P[4] disease and there was no difference in risk of P[8] disease [13]. Among the unvaccinated in that trial, Lewis-negative infants tended to be at lower risk of P[8] disease, and were at significantly increased risk of P[6] disease (these associations have also been described for children from Burkina Faso [5]). Within the RV1-vaccinated arm in Bangladesh, those similar associations were found between the infants' HBGA status and postvaccination risk of rotavirus disease by specific P-genotypes. As the authors comment, their finding of similar risk of P[8] disease in nonsecretors vs secretors may suggest that unique strains of P[8] may differ in their ability to infect nonsecretors (eg, the G9P[8] strain in their study) [13]. In addition to human rotavirus infectivity and glycan binding investigations using MA104 cells [14], the auspicious work with human intestinal enteroids may further reveal if there are differences in host restriction, via HBGAs and beyond, between wild-type and attenuated human rotavirus vaccine strains of the same genotype, as well as mechanisms of vaccine strain attenuation [15].

In Ghanaian infants, we did not find an association with salivary ABO status; as with other evaluations that have reported results on this aspect, our study was not specifically powered to examine this possibility. Our results, however, are different from the Pakistan findings, where secretors of blood group type O (by salivary antigen) had statistically higher likelihood of seroconversion compared with secretors of non-blood group O (RR, 1.7 [95% CI, 1.1–2.7]). In Nicaraguan infants, among secretors, those of blood type B (by hemagglutination) were the

group with the lowest frequency of increased antirotavirus IgA titer after RV1 dose 1 [12]. In vitro data have suggested that the type B epitope may interfere with the binding of P[8] strains [1].

Our study has limitations. HBGA phenotype in saliva, as an indicator of that expressed at the gastrointestinal mucosal surface, was determined from saliva collected at age 4 years and not at time of vaccine receipt in early infancy, which could be relevant if there are phenotype changes during this time. We did not perform genotyping for all subjects to allow full comparison of results using phenotype only vs genotype only. However, we had high concordance between secretor phenotype and genotype and our findings were consistent when we incorporated available genotype results or when phenotype only was used. Inherent in nearly all rotavirus vaccine trials, seroconversion in some of our infants may reflect wild-type infection rather than vaccine response.

Our data support the theory that secretor status plays a role in host susceptibility to rotavirus infection, specifically from P[8] genotypes. Continued laboratory advancements and vaccine studies that incorporate HBGA assessments will be important to understand the extent to which such host factors impact our measurements of rotavirus vaccine performance as well as risk for possible adverse events (ie, intussusception) in different populations.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The findings and conclusions of in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

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